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Ingestion of Ultraplankton by the Planktonic Larvae of the Crown-of-Thorns Starfish, *Acanthaster planci*

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Abstract. There has been a debate over whether the growth and development of the larvae of the crown-of-thorns starfish *Acanthaster planci* are severely food-limited. This debate has raised a range of questions, including the one relating to the role of heterotrophic bacteria in the nutrition of larvae. In this study, the feeding rate of larvae on bacteria as well as on other ultraplankton (<5 μm) was determined by counting the number of the fluorescence-labeled cells (FLC) in the gut after short incubation. Preliminary experiments showed no detrimental effect of the fluorescence dye (5-(4,6-dichlorotriazin-2-yl) aminofluorescein) on the development of larvae and demonstrated the usefulness of FLC in feeding experiments as food particles analogous to living cells of ultraplankton. There was no evidence that larvae ingested bacteria. Larvae did ingest two strains of photosynthetic cyanobacteria, which had equivalent spherical diameters (ESD) of 1 and 1.8 μm, but these tiny cells were cleared more than 10 times slower than the larger algae *Phaeodactylum tricornutum* (4.7 μm ESD) and *Dunaliella tertiolecta* (5.1 μm ESD). Regardless of the size of FLC used, the clearance rate (volume of water cleared per animal per unit time) increased by 50–120%, as larvae developed from the late bipinnaria stage to the late brachiolaria stage. These results show that larvae may derive a sizable proportion of their nutrition from ultraplankton, but not from bacteria.

Introduction

Heterotrophic bacteria make up a large, metabolically active component of plankton assemblages in the sea (*e.g.*, Pomeroy, 1974; Azam *et al.*, 1983). Bacterial biomass is usually equal to 10–40% of phytoplankton biomass (*cf.* Ducklow, 1983), but can be greater than phytoplankton biomass in oligotrophic waters (Fuhrman *et al.*, 1989; Cho and Azam, 1990). Furthermore, the proportion of ultraplankton (<5 μm, Murphy and Haugen, 1985) in phytoplankton assemblages reaches more than 50% in a wide range of environments (*cf.* Stockner, 1988). Use of these predominant, small particles as a food source is thought to be enormously advantageous for planktonic larvae surviving under nutrient-impoverished conditions, but their actual use by asteroid larvae has been a matter of controversy over the past decade. Specifically, bacteria were ingested by antarctic asteroid larvae (Rivkin *et al.*, 1986; Pearse *et al.*, 1991), but not by temperate ones (Pearse *et al.*, 1991).

The crown-of-thorns starfish (COTS) *Acanthaster planci* is unique in tropical and subtropical waters. Gut content analysis with a scanning electron microscope (SEM) has shown that the natural diet of COTS larvae almost exclusively comprises relatively rare, large phytoplankton, such as dinoflagellates and pennate diatoms of up to 200 μm in length (P. Dixon, unpub.). Dixon's results have supported the idea that, except during occasional phytoplankton blooms, the availability of the preferred phytoplankton to COTS larvae is very low and therefore the growth and development of COTS larvae are food-limited (Lucas, 1982). In contrast, experiments in which COTS larvae were reared in *in situ* chambers showed no evidence of food limitation under the low phytoplankton conditions common in tropical waters (Olson, 1985, 1987). Results from the rearing studies have been interpreted as indirect evidence that COTS larvae can exploit non-phytoplankton food, including bacteria and dissolved organic matter (Olson and Olson, 1989; Birkeland and Lucas, 1990).

Bacteria were not positively identified in the gut contents of COTS larvae (Dixon, unpub.). However, this may be due to the difficulty in finding bacteria in various food

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remains or the loss of bacteria during a number of processes involved in the SEM sample preparation. Sherr *et al.* (1987) determined the feeding rate of heterotrophic nanoflagellates on bacteria by directly counting the number of heat-killed, DTAF (5-(4,6-dichlorotriazin-2-yl) aminofluorescein)-labeled cells in food vacuoles. The yellow-green fluorescence of DTAF-labeled cells is distinctive and intense, so that this technique has also been used to study the feeding of larger protozoans and rotifers (Rublee and Gallegos, 1989).

The first objective of this study was to test the feasibility of the fluorescence-labeled cell (FLC) technique for determining the feeding rate of COTS larvae on ultraplankton. The possibility of differences in chemical properties between living and heat-killed cells (Paffenhofer and Van Sant, 1985) made it particularly important to examine whether COTS larvae ingest FLC at similar rates to living cells. The second objective was to use the FLC technique to determine the feeding rate of COTS larvae on bacteria and other ultraplankton.

Materials and Methods

COTS larvae were grown in the mass rearing facility at the Australian Institute of Marine Science, using mixtures of three cultured phytoplankton, *Dunaliella primolecta, D. tertiolecta,* and *Phaeodactylum tricornutum.* Because larvae in the tanks were at different developmental stages (the distinction criteria described by Lucas, 1982), about 600–800 larvae were individually sorted from the mass-reared populations, placed in 250-ml bottles (<150 larvae per bottle) filled to the top with filtered seawater, and held without food. After 24 h, only actively swimming larvae in the top quarter of each bottle were removed and used for experiments. This procedure was designed to reduce the chance of using physiologically retarded larvae. Seawater used in experiments was obtained at the rearing facility and filtered through 0.45-μm Millipore filters just before use. Experiments were run at 28°C under dim light.

FLC preparation

Six types of FLC were prepared: three cultured algal species (*P. tricornutum, D. tertiolecta,* and *Tetraselmis* sp); two cultured cyanobacteria species (Strain ACMM326, the culture collection of the Sir George Fisher Centre, James Cook University of North Queensland, and an unidentified strain); and natural bacteria. These cells were heat-killed and stained with the fluorochrome DTAF (Sigma #D2281). Methods were as described by Sherr *et al.* (1987), except for minor modifications to the speed and duration of centrifugation of algal suspensions (1500 rpm for 10 min for *P. tricornutum* and *D. tertiolecta,* 800 rpm for 10 min for *Tetraselmis* sp., 6000 rpm for 15 min for cyanobacteria) and addition of a pre-wash with a 1.5% NaCl solution before incubation in a DTAF solution.

Toxicity of FLC

To test the toxicity of FLC, larvae were allowed to feed on fluorescence-labeled *P. tricornutum* for 2 days and their survival and development were examined. At the beginning, 50 larvae at the early brachiolaria stage were introduced into each of ten 100-ml bottles (five bottles per treatment) filled to the top with a labeled or a living (as control) cell suspension at about 1000 cells ml^{-1}. These bottles were placed on a cell shaker (30 shakes min^{-1}) to keep cells in suspension and incubated at 28°C under an 8 h light-16 h dark cycle.

Gut filling rate

Two conditions must be fulfilled for the FLC method to provide reliable feeding rate estimates: the feeding rate must be constant and the incubation must be short enough that no defecation occurs. Then, providing that the change in FLC concentration during incubations is negligible, the clearance rate (volume of water cleared per animal per unit time) can be calculated by dividing the gut filling rate (cells per animal per unit time) by the FLC concentration.

To ensure these conditions were met, the gut filling rate was determined by measuring the number of FLC in the gut over time. After 24 h starvation, 15 larvae at the early brachiolaria stage were introduced into each of 21 scintillation vials (3 vials per treatment) with 20 ml of filtered seawater, and 200 μl of a labeled *P. tricornutum* suspension was added (the final concentration was 806 cells ml^{-1}). After 2- to 30-min incubations, 1 ml of a 10% buffered glutaraldehyde solution was added to the vials. Preserved samples were then refrigerated for later FLC counting. DTAF fluorescence did not fade out during several weeks of cool storage.

Preserved larvae were examined under a Zeiss epifluorescence microscope equipped with a blue excitation filter set (#487909). Before counting, preserved larvae were poured into plastic tubing with a 80-μm-mesh screen at one end and gently washed with filtered seawater. A small amount of water with larvae was then filtered onto 0.45-μm Millipore filters stained with Irgalan black. After excess seawater was removed, filters with larvae were mounted on glass slides, using a Zeiss immersion oil (#58884). The number of FLC in the gut was counted for 10 larvae in each vial.

Discrimination between FLC and living cells

In this experiment, larvae at the late bipinnaria and early and late brachiolaria stages were allowed to feed on

Table I

Size of algal and bacterial cells used for feeding experiments

Type	L (μm)	ESD (μm)
Phaeodactylum tricornutum	23.9	4.7
Dunaliella tertiolecta	5.6	5.1
Tetraselmis sp.	27.5	17.2
Cyanobacteria, ACMM326	2.2	1.8
Cyanobacteria—small strain	1.0	1.0
Natural bacteria	0.2–0.8	—

L: Length of long axis, ESD: equivalent spherical diameter.

mixtures of FLC and living cells of the algae *P. tricornutum*, *D. tertiolecta*, and *Tetraselmis* sp. and two species of cultured cyanobacteria at various proportions for 5–10 min. FLC and living cells in the gut were counted immediately after experiments, because the autofluorescence of living cells faded rapidly when stored.

Feeding rate

FLC uptake was determined for larvae at the late bipinnaria and early and late brachiolaria stages. Fifteen larvae were introduced into each experimental vial with 20 ml of filtered seawater. After about 2 h, 80–2000 μl of FLC suspension was added to each vial. As described below, larvae ingested FLC selectively over living cells of *Tetraselmis* sp. Therefore, *Tetraselmis* sp. was no longer used in this series of experiments. The concentration range of FLC was 0.55–5.37 $\times 10^3$ cells ml^{-1} for *P. tricornutum*, 0.12–7.46 $\times 10^3$ cells ml^{-1} for *D. tertiolecta*, 0.31–1.27 $\times 10^4$ cells ml^{-1} for cyanobacteria ACMM326, 0.43–10.90 $\times 10^4$ cells ml^{-1} for unidentified cyanobacteria, and 0.78–3.92 $\times 10^5$ cells ml^{-1} for natural bacteria. As shown in Table I, the equivalent spherical diameter (ESD) ranged between 1 and about 5 μm. The duration of incubation was chosen between 3 and 10 min., depending on the type and concentration of FLC. Samples obtained were preserved and processed as described in the measurements of gut filling rates.

Results

Toxicity of FLC

No larvae died during 2-day incubations in either FLC or control treatment, although a few deformed larvae were observed. All larvae observed under an epifluorescence microscope had living cells or FLC of *P. tricornutum* in the gut. In the FLC treatment, the gut walls of larvae were stained with DTAF, indicating that larvae had assimilated DTAF-labeled cell protein.

Gut filling rate

The number of labeled *P. tricornutum* cells in the gut increased linearly with time and did not level off within 20 min (=0.33 h) (Fig. 1). The clearance rate calculated for this time interval was 0.38 ml ind^{-1} h^{-1} (=[302 cells ind^{-1} h^{-1}]/[806 cells ml^{-1}]). The results of 25- and 30-min incubations are not shown because the number of cells in the gut exceeded 100 cells and FLC counting became difficult. Incubation time, although dependent on the type and concentration of FLC, should not exceed 20 min. Generally, 5-min incubations were long enough to determine the feeding rate of COTS larvae.

Discrimination between FLC and living cells

The proportion of FLC to living cells in the gut was plotted against that in experimental medium and a line representing the 1:1 ratio was drawn (Fig. 2). The points for *P. tricornutum*, *D. tertiolecta*, and two strains of cyanobacteria were around the 1:1 line, suggesting that larvae nonselectively ingested living and DTAF-labeled cells of these small algae. On the other hand, the points for *Tetraselmis* sp. deviated upward from the 1:1 line, suggesting selective ingestion of FLC over living cells. The difference between clearance rates of larvae on living cells and FLC was consistently highly significant for *Tetraselmis* sp. (Student's *t*-test, $P < 0.001$ or $P < 0.0001$), but not for other algae (Table II).

Feeding rate

Labeled bacteria were ingested very occasionally by larvae of all three developmental stages examined. Bacteria observed in the gut were usually clumped together and often seen attached to flocculent material. A small

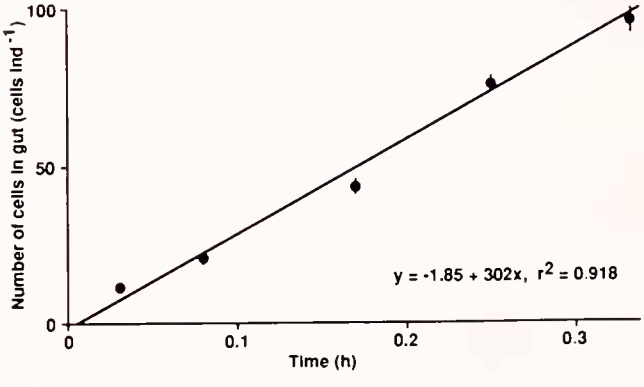

Figure 1. Time-course of the increase in the cell number in the gut of early brachiolaria larvae of *Acanthaster planci* feeding on heat-killed, fluorescence-labeled cells of *Phaeodactylum tricornutum* at a concentration of 806 cells ml^{-1}.

amount of such clumped material was also seen in original FLC solutions. Thus larvae were probably ingesting clumped material rather than individual cells.

The rates of ingestion and clearance of all FLC except *Tetraselmis* sp. by larvae at the late bipinnaria and early and late brachiolaria stages are shown in Figures 3–5. In all experiments, the ingestion rate increased linearly with FLC concentration and there was no evidence of saturation of ingestion rates. The 95% confidence bands of the regression line, although not shown, covered the origin. Thus the clearance rate remained constant over the range of FLC concentrations tested.

The clearance rate of larvae increased by almost two orders of magnitude as the ESD of FLC increased from 1 to 5 μm (Fig. 6). The clearance rate also increased with larval age: from the late bipinnaria to the late brachiolaria stage, the rate increased by 120% for *P. tricornutum*, 80% for *D. tertiolecta*, 50% for the small cyanobacteria strain, and 70% for cyanobacteria ACMM326.

Discussion

Feasibility of the FLC technique

The FLC technique demonstrates both strengths and weaknesses when applied to the feeding of asteroid larvae. With larger food particles, the usefulness of the method is limited, as suggested by the selective ingestion of FLC over living cells of *Tetraselmis* sp. by COTS larvae. For ultraplankton-sized algae, however, this technique has an obvious advantage over the conventional cell counting method. Generally, the feeding rate of zooplankton is determined by measuring the change in cell concentration during incubations (Frost, 1972). This method, because of the low precision and accuracy of cell counting, is inadequate to detect small changes in cell concentration and thus cannot be used for determining the feeding rate of individuals. This problem is particularly serious for small-sized algae, making it impossible to determine the lower size limit of food particles with the cell counting method. DTAF fluorescence, on the other hand, is very intense and clearly visible even through the gut wall of COTS larvae (Fig. 7), leaving little chance for counting errors.

Although some conditions can interfere with the accuracy of FLC counts, these usually can be overcome or avoided. For example, FLC in the gut can densely overlay each other, depending on the orientation of body on filters. This problem can be solved simply by excluding such individuals from counting. Another potential problem, accumulation of FLC in the posterior part of the esophagus, did not become serious within the range of FLC concentrations in the present feeding rate measurements.

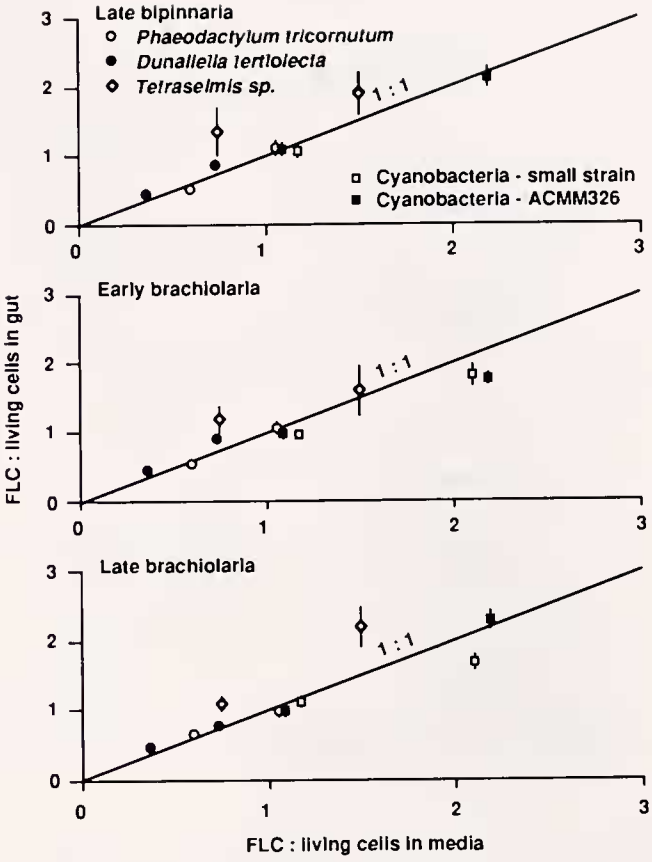

Figure 2. Comparison of the proportion of heat-killed, fluorescence-labeled cells to living cells between experimental media and the gut content of the larvae of *Acanthaster planci*.

Direct count of algal cells in the gut of echinoderm larvae is not new (Strathmann, 1971), but has been limited by the resolution of light microscopy. The use of an epifluorescence microscope in feeding studies promises more reliable data with less effort and time. The autofluorescence of algal cells, if samples are examined immediately after fixation, can be seen in the gut. DTAF-labeled cells produce bright yellow-green fluorescence at the broad blue excitation band (450–490 nm) of the Zeiss filter set used. The autofluorescence of two strains of phycoerythrin-rich cyanobacteria is orange-yellow and more or less similar to DTAF fluorescence. Nevertheless, the DTAF fluorescence can be distinguished easily from the autofluorescence of cyanobacteria, unless the gut becomes congested with cells.

Selective ingestion of FLC over living cells of *Tetraselmis* sp. by COTS larvae may be ascribed to the inefficient capturing of the motile cells of *Tetraselmis* sp. Alternatively, living cells of *Tetraselmis* sp. may release chemical compounds that COTS larvae do not favor. The possibility of such chemosensory feeding has been sug-

Table II

Two-sample t test for the difference between mean clearance rates of the larvae of Acanthaster planci *on living (C_{live}) and heat-killed, fluorescence-labeled cells (C_{FLC}) of five algae*

	C_{live} (ml ind^{-1} h^{-1})	C_{FLC} (ml ind^{-1} h^{-1})	C_{live} *vs.* C_{FLC}
Phaeodactylum tricornutum			
Late bipinnaria	2.64×10^{-1} (0.09×10^{-1})	2.30×10^{-1} (0.11×10^{-1})	>*
Early brachiolaria	2.91×10^{-1} (0.08×10^{-1})	2.52×10^{-1} (0.11×10^{-1})	>*
Late brachiolaria	3.23×10^{-1} (0.11×10^{-1})	3.28×10^{-1} (0.16×10^{-1})	<
Dunaliella tertiolecta			
Late bipinnaria	2.39×10^{-1} (0.12×10^{-1})	2.62×10^{-1} (0.12×10^{-1})	<
Early brachiolaria	2.45×10^{-1} (0.11×10^{-1})	3.03×10^{-1} (0.13×10^{-1})	<*
Late brachiolaria	2.90×10^{-1} (0.08×10^{-1})	3.11×10^{-1} (0.13×10^{-1})	<
Tetraselmis sp.			
Late bipinnaria	1.68×10^{-1} (0.11×10^{-1})	2.56×10^{-1} (0.13×10^{-1})	<***
Early brachiolaria	3.01×10^{-1} (0.15×10^{-1})	3.94×10^{-1} (0.25×10^{-1})	<**
Late brachiolaria	4.50×10^{-1} (0.19×10^{-1})	6.45×10^{-1} (0.21×10^{-1})	<***
Cyanobacteria—small strain			
Late bipinnaria†	5.87×10^{-3} (0.51×10^{-3})	5.76×10^{-3} (0.27×10^{-3})	>
Early brachiolaria	6.89×10^{-3} (0.34×10^{-3})	6.00×10^{-3} (0.21×10^{-3})	>
Late brachiolaria	7.26×10^{-3} (0.29×10^{-3})	5.92×10^{-3} (0.24×10^{-3})	>*
Cyanobacteria—ACMM326			
Late bipinnaria	1.35×10^{-2} (0.05×10^{-2})	1.31×10^{-2} (0.06×10^{-2})	>
Early brachiolaria	1.68×10^{-2} (0.07×10^{-2})	1.40×10^{-2} (0.05×10^{-2})	>*
Late brachiolaria	1.85×10^{-2} (0.08×10^{-2})	1.93×10^{-2} (0.09×10^{-2})	<

Numbers in parentheses are standard errors. The number of larvae for each mean value is 20, except for one case (†) where $n = 10$.
* $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$.

gested for other asteroid larvae, despite their simple feeding mechanism with a single band of cilia (Strathmann, 1971; Strathmann *et al.*, 1972). It is also known that asteroid larvae show very sophisticated feeding behaviors; *i.e.*, selective ingestion of the most abundant particles from time to time (Rassoulzadegan and Fenaux, 1979). Even if COTS larvae possess chemosensory organs, however, the chemical stimuli from ultraplankton-sized algae are seemingly too weak to elicit the selective feeding response of COTS larvae. Otherwise, COTS larvae would ingest living and heat-killed cells of *P. tricornutum*, *D. tertiolecta*, and two strains of cyanobacteria at different rates. Overall, the FLC technique seems very useful in determining the feeding rate of asteroid larvae on ultraplankton-sized food particles.

Ingestion of bacteria by COTS larvae

Fluorescence-labeled bacteria were not found in the gut of COTS larvae. One of the aspects that were not fully covered in this study is ingestion of bacteria attached on detritus. In reef waters, a large amount of detritus, such as coral mucus, benthic algal fragments, and fecal matter, is released into the water column. Dense colonies of bacteria are often observed on this detritus, particularly on coral mucus (*e.g.*, Ducklow and Mitchell, 1979; Rublee *et al.*, 1980). Up to 50% of bacteria are reported to be associated with detritus in reef waters (Sorokin, 1974; Moriarty, 1979). Attached bacteria are thought to account for 20–30% of the total bacterial biomass in other environments (Sorokin, 1981), although separation of free-living bacteria from attached bacteria by filters or mesh screens is problematic.

Strathmann *et al.* (1972) used high-speed cinematography to show that marine invertebrate larvae with a single band of cilia (including asteroid larvae) capture particles by reversing the beat of several cilia. Gilmour (1988) also reported that asteroid larvae capture particles by direct interception on their cilia. Results of both cinematographic studies indicate that the mechanical disturbance caused by very small particles like bacteria is not strong enough to trigger such ciliary action effectively.

The present observation that COTS larvae are not able to ingest bacteria agrees with results reported for the larvae of the temperate starfish *Asterina miniata* (Pearse *et al.*, 1991), but not for those of the antarctic starfish *Porania antarctica* (Rivkin *et al.*, 1986) and *Odontaster validus* (Pearse *et al.*, 1991). Rivkin *et al.* (1986) reported that the larvae of *P. antarctica* selectively ingested bacteria in bacteria-phytoplankton mixtures. Pearse *et al.* (1991) not only substantiated the results of Rivkin *et al.*, using the larvae of *O. validus*, but also reported that the larvae of

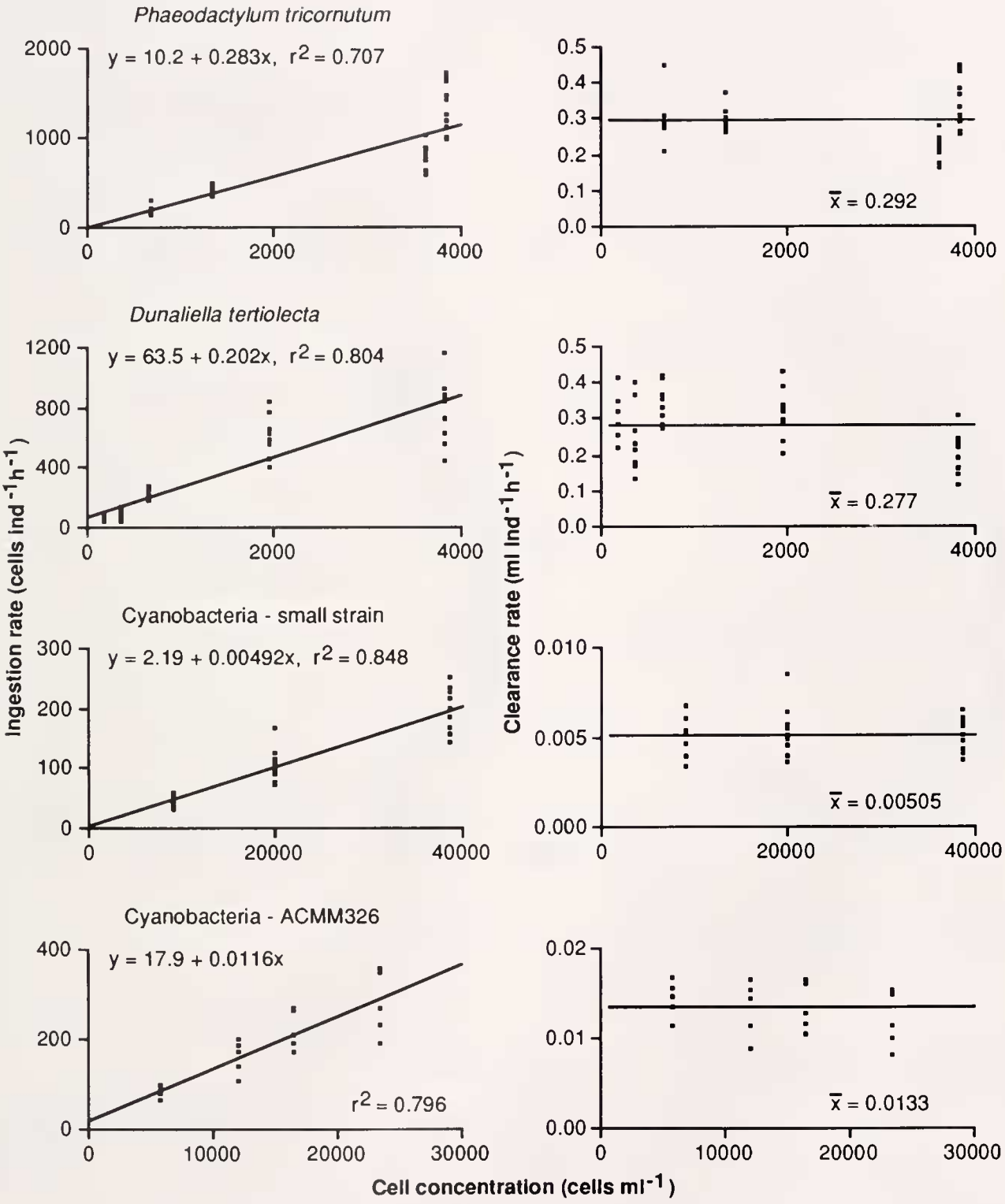

Figure 3. Ingestion and clearance rates of late bipinnaria larvae of *Acanthaster planci* feeding on four types of fluorescence-labeled cells.

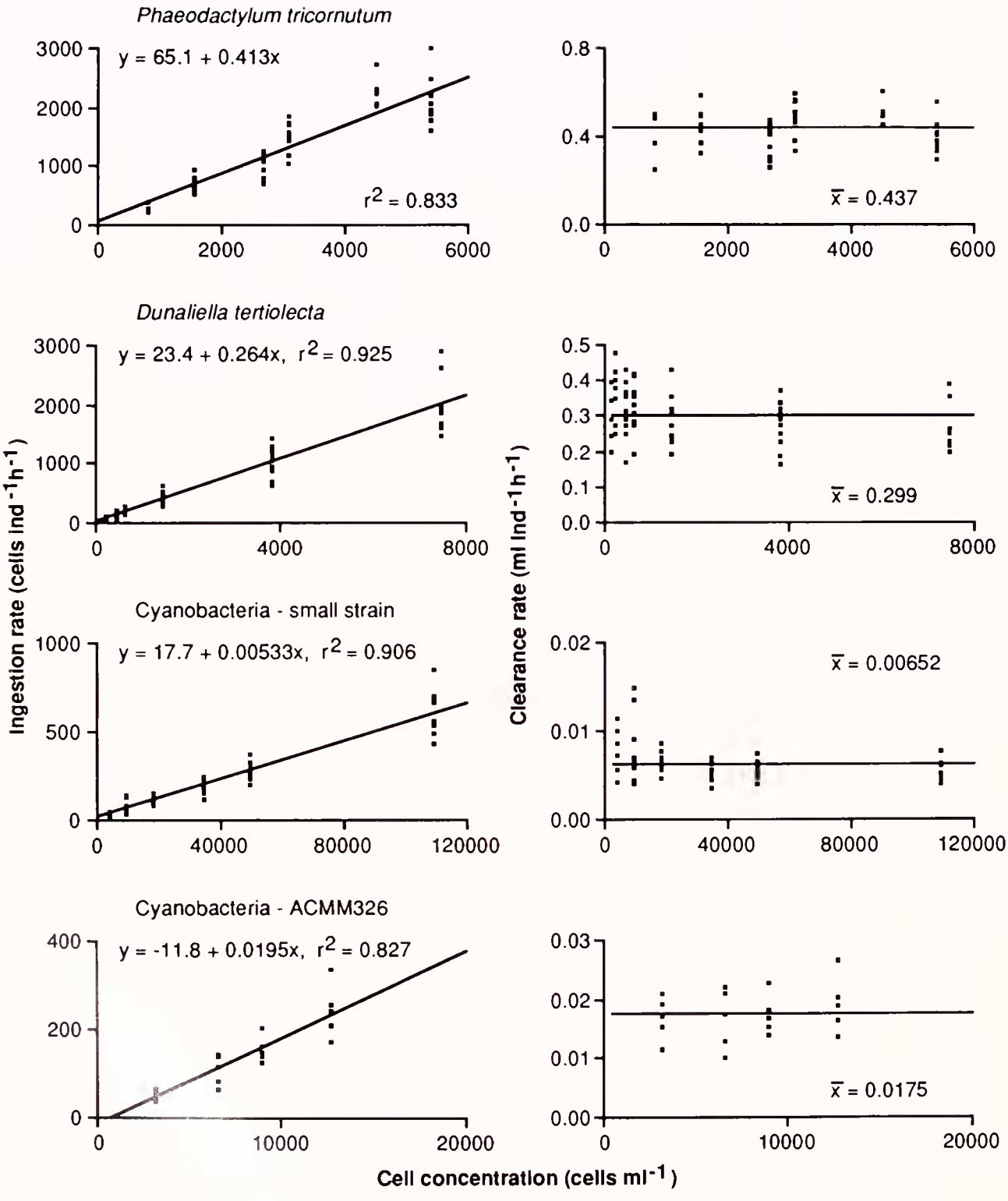

Figure 4. Same as Figure 3, but for early brachiolaria larvae of *Acanthaster planci*.

Late brachiolaria

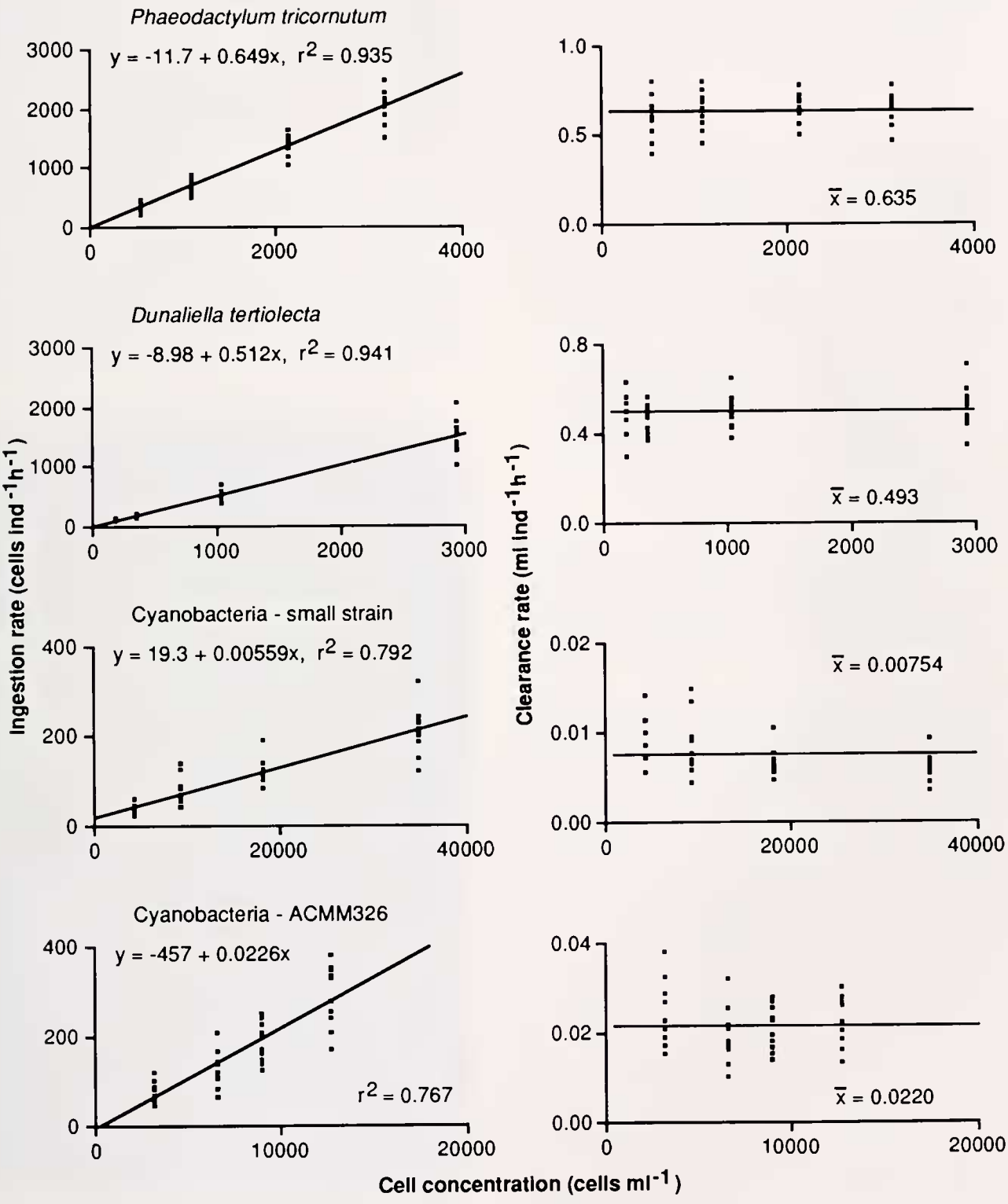

Figure 5. Same as Figure 3, but for late brachiolaria larvae of *Acanthaster planci*.

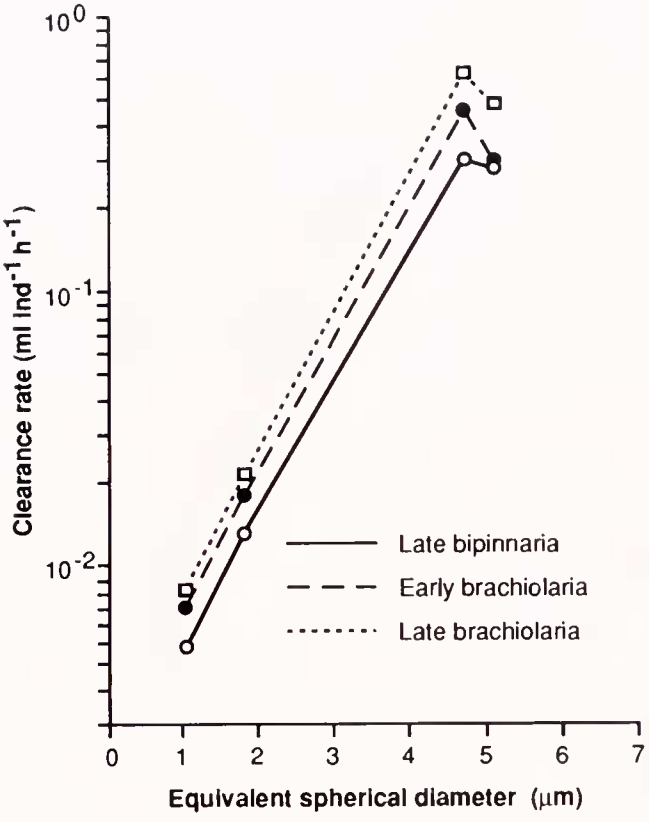

Figure 6. Effect of algal cell size on the clearance rate of the larvae of *Acanthaster planci* at three developmental stages.

O. validus grew equally well when reared on bacteria alone or on bacteria-phytoplankton mixtures. In contrast, the same authors, using the same methods, found no significant ingestion of bacteria by the larvae of *A. miniata*. One explanation for these contradictory results is that antarctic asteroid larvae are adapted to their rather unique environments and have a particle-capturing mechanism different from those of temperate and tropical species, despite their resemblance in morphology. It is also necessary to consider the possible difference in the proportion of attached to free-living bacteria between experiments. As mentioned above, this study does not deny the likelihood of ingestion of attached bacteria by asteroid larvae.

Ingestion of ultraplankton by COTS larvae

In the present experiments, COTS larvae were able to ingest two strains of cyanobacteria, but not bacteria, indicating that about 1 μm represents the minimum size of food particles for COTS larvae. Another important finding is the rather phenomenal increase in clearance rate between particles of 1 and 5 μm ESD: COTS larvae ingested *P. tricornutum* and *D. tertiolecta* more than ten times faster than they did two strains of cyanobacteria.

The majority of ultraplankton bigger than cyanobacteria consist of different forms of flagellates. Cyanobacteria are usually the dominant component of ultraplankton in shelf waters of the Great Barrier Reef (Furnas and Mitchell, 1986). In coral reef lagoons, however, flagellates make a significant contribution to ultraplankton biomass (Ayukai, 1992) and are a potentially important food source for COTS larvae. Although the results of the gut content analysis (Dixon, unpub.) do not support this possibility, flagellates in this size class are extremely fragile and could have been lost during sample fixation and preparation for SEM.

An increase in cell size beyond the ultraplankton size range appears to have relatively little effect on the clearance rate of COTS larvae. For instance, the clearance rates on FLC of *Tetraselmis* sp. (17.2 μm ESD) were similar

Figure 7. Heat-killed, fluorescence-labeled cyanobacteria (arrows, *ca* 1 μm in diameter) in the gut of brachiolaria larva of *Acanthaster planci*. The autofluorescence of live cells of *Phaeodactylum tricornutum* (pennate) and *Dunaliella tertiolecta* (oval) is also seen in the background.

to those on FLC of *P. tricornutum* and *D. tertiolecta* at bipinnaria and early brachiolaria stages and only two times higher than those on FLC of *P. tricornutum* and *D. tertiolecta* at late brachiolaria stage. The selective feeding of larvae on FLC over living cells of *Tetraselmis* sp. suggests that factors other than cell size (*e.g.*, cell shape, motility, chemical properties) may become more important to COTS larvae capturing larger particles.

The ingestion rate of COTS larvae in this study increased linearly with increasing FLC concentration and the clearance rate remained constant, independent of FLC concentration. This result contradicts the previous study by Lucas (1982), who reported a decrease in clearance rate with increasing cell concentrations. For instance, the clearance rate for *D. tertiolecta* in this study is comparable to his values for *D. primolecta* at low cell concentration (<1000 cells ml^{-1}), but is clearly different at higher concentrations (1000–4000 cells ml^{-1}). This is probably because feeding rates determined using the FLC method better reflect the efficiency of food particle capture by the band of cilia than do rates based on the conventional method, which can be affected by physiological and behavioral factors. As observed under an epifluorescence microscope, the turnover of cells in the gut of COTS larvae is relatively slow. Although the gut is soon congested at high cell concentrations, COTS larvae continue to capture cells, rejecting excess cells in the esophagus by the dorsal flexion of the body (Lucas, 1982). In long incubations at high cell concentrations, it is quite likely that COTS larvae capture many more cells than they actually ingest.

Body length approximately doubles, from about 0.7 to 1.4 mm, as COTS larvae progress from the late bipinnaria stage to the late brachiolaria stage. In this study, the clearance rates of larvae on four types of FLC also increased by 50–120% during this period. This result agrees with a general trend that the clearance rate of planktonic larvae with a single band of cilia is largely proportional to the length of the ciliated band (Strathmann *et al.*, 1972; Strathmann, 1975), which in turn depends on body length.

At present, little is known about the nutritional value of ultraplankton for the growth, development, and survival of asteroid larvae. The variability of the size frequency distribution and biomass of ultraplankton in putative habitats of different asteroid larvae is also unknown. The results presented here should not be extrapolated directly to other asteroid larvae, but do suggest that a significant proportion of ultraplankton is a potential food source for asteroid larvae.

Acknowledgments

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